Cloning and expression of *Hsp*22.4 gene from *Chaetomium globosum*

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Abstract: A study was conducted on the molecular mechanism of small heat shock proteins (*sHSPs*) in *Chaetomium globosum*. Heat shock protein 22.4 (*Hsp*22.4) from *C. globosum* was cloned and expressed in *Escherichia coli*. BlastX analysis revealed that the *Hsp*22.4 gene from *C. globosum* shared the highest identity in amino acid sequence with a *Hsp* gene from *Neurospora crassa*, and the identity between them was 65%. The *C. globosum Hsp*22.4 gene was inserted into the expressive vector of pGEX-4T-2 and the recombinant plasmid named pGEX-HSP. *E. coli* BL21 transformed with pGEX-HSP plasmid was induced by IPTG, and the expressed proteins were analyzed with SDS-PAGE. A 50 kD protein was specially expressed in *E. coli* BL21, and the result was consistent with expectation, and showed that the *Hsp*22.4 gene had been expressed in *E. coli.* Our study has made a foundation for further studying the function of *sHSPs* protein.

Keywords: *Chaetomium globosum*; Heat shock proteins (*HSPs*); Gene cloning and Expression
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Introduction

Heat shock proteins (*Hsps*), a protein family, can be induced by cell stimuli-responses with a function of molecular chaperone, which can prevent the accumulation of protein precursor, accelerate the transport of protein, absorb complex with unfolded proteins to maintain their transport abilities, sustain the normal fold state of protein, help to degrade misfolding protein, stabilize polypeptide strand, and prevent protein inactivity under the conditions of various stimulus. *Hsps* also participate in the regulation for the activation and function of target proteins, although they are not the components of the target proteins. *Hsps* with low molecular mass (about 15 to 30 kD) are called small heat shock proteins (*sHsps*) (Sugiyama *et al*. 2000). *sHsps* family is a stress-inducible group of molecular chaperones and can prevent the polymerization of denatured protein. In *Bradyrhizobium japonicum*, alterable amino acid sequences of *sHsps* exert its function through high conservative G114 glycine in alphaB-crystallin protein region (Lentze *et al*. 2003). *Hsp* 16.3 in *Mycobacterium tuberculosis* could modulate its chaperone activity by adjusting the dynamic of oligomeric dissociation/re-association with substrates, while its static oligomeric size maintains invariability (Fu *et al*. 2004).

Chaetomium globosum, a kind of important bio-control fungus, can produce many kinds of antibiotics and ergosterols to inhibit the growth of pathogens (Kanokmedhakul *et al*. 2002; Reissinger *et al*. 2003). At present, many studies have been mostly focused on physiology of *C. globosum* and its application in bio-control, however, few studies have been done on bio-control mechanism at the gene level. On the basis of the partial sequence of *Hsp*22.4 obtained from *C. globosum* cDNA library, we have cloned the full length of cDNA sequence of *Hsp*22.4 gene from *C. globosum*, and insert this gene into the pGEX-4T-2 vector. The prokaryotic expression was performed, and *Hsp*22.4 gene was successfully expressed in *Escherichia coli*. This study will provide a basis for studying the mechanism of response to environments stress in *C. globosum.*

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Materials and methods

Bacterial strains and plasmid

Spores of the *C. globosum* strain were put into PD liquid medium and incubated at 25°C, 220 rpm for 36 h, and then were heat shocked at 40°C, 220 rpm for 30 min. Mycelium were collected by centrifuging at 4 000 rpm for 10 min and immediately frozen in liquid nitrogen, then stored at –70°C.

Extraction total RNA and synthesis single-strand cDNA

Total RNA was extracted from mycelium and purified according to Wang's protocol (Wang *et al*. 2003). Total RNA was treated with DNase (RNase free, promega) at 37°C for 30 min, then extracted by phenol and chloroform, and precipitated by alcohol. Single-strand cDNA was synthesized from 1 μg of total RNA at 42°C for 60 min with 200 U of reverse transcriptase (Superscript II, invitrogen). The synthesized single-strand cDNA were acted as template for amplifying *Hsp* 22.4 gene form *C. globosum*.

Cloning of *Hsp* 22.4 gene and Construction of prokaryotic expression vector

For cloning of *Hsp* 22.4 gene and inserted it into pGEX-4T-2 vector for expression in *E.coli*. Primer for amplifying open reading frame of *Hsp* 22.4 gene were designed according to the sequence of *C. globosum Hsp* 22.4 gene (AY491980), Primer HSPL:
5′-GACATGGATCCATGTCCTTCTTCACTCGTGGCCTT-3′
(*Bam* HⅠ site in underline); primer HSPR:
5′-TAATGCTCGAGTTAGTTGATAGCAACACGGCGCGC-3′
(*Xho* Ⅰ site in underline). 20 μL reaction system contained 2 μL 10×EX *Taq* PCR Buffer; 0.4 μL dNTP (10 mmol·L^{-1}); 0.5 μL primer HSPL (20 μmol·L^{-1}), HSPR (20 μmol·L^{-1}), 1 μL template cDNA, 2 U EX *Taq*, and added ddH_2O to 20 μL. PCR program is: 120 s at 94 °C; 20 s at 94 °C, 30 s at 64°C, 90 s at 72 °C for 30 cycles. After the last cycle, the amplification was extended to 10 min at 72°C. The amplified products were separated by agarose gel electrophoresis and cloned into cloning vector of pMD18-T (TaKaRa), and then sequenced.

The above amplification product and vector pGEX-4T-2 (Pharmacia) were digested by *Bam* HⅠ and *Xho* Ⅰ, respectively, double enzymes digested products of amplification product and vector DNA were purified, ligased and transformed into *E. coli* DH5α. Positive clones were screened in LB medium containing 50 mg··L^{-1} kanamycin. After transformation, two transformants, named pGEX-HSPa and pGEX-HSPb, were screened by PCR using primers HSPL and HSPR and double enzyme digestion using *Bam* HⅠ and *Xho* Ⅰ.

Induced expression of recombinant vector pGEX-HSPb

Recombinant vector pGEX-HSPb and the control (vector pGEX-4T-2) were transformed into *E. coli* BL21 for the analysis of protein expression. After incubating 12 h at 37 °C in LB medium, cells were diluted 50 times, when the OD_{600} of cells reached 0.4-0.6 at 37°C, adding IPTG to final concentration of 1.0 mmol·L^{-1} immediately, then induced 30 min at 30°C, and collected separately *E. coli* cells at 3 h, 4 h, 5 h and 6 h. The collected cell were added with 1×loading buffer, boiled 5 min, and centrifuged 10 min at 8 000 rpm, then the supernants were loaded onto the slab gel of 12% SDS-PAGE. The gel was stained with Coomassie Brilliant Blue G-250.

Bioinformatic analysis of *Hsp*22.4 gene

Multiple alignment of amino acid sequence of *Hsp*22.4 gene from different organism was conducted. *Hsp*22.4 protein from *C. globosum* (AAR36902), *Neurospora crassa* (XP_332056), *Aspergillus nidulans* FGSC A4 (EAA59763), *Emericella nidulans* (P40920), *A. oryzae* (BAD02411) and *Exophiala dermatitidis* (AAL84791) were aligned by ClustalX program. Phylogenetic tree was drawn by ClustalX program (1.8).

Results

Total RNA extraction and its quality identification

Result of agarose gel electrophoresis showed that there were two brightness bands: 28S rRNA and 18S rRNA, and the brightness of 28S rRNA is twice than that of 18S rRNA (Fig. 1). The ratio of A_{260} to A_{280} is 1.9, which proved that the RNA quality reached the standard.

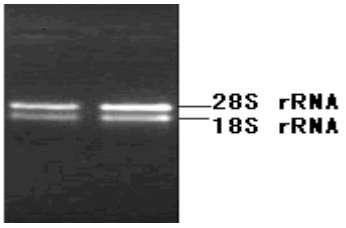

Fig. 1 The electrophoresis pattern of total RNA

Analysis of sequence identity

Multiple sequence alignment proved that the identities between amino acid sequence of *sHsp*s genes from *C.globosum* and *N.crassa* (XP_332056), *A. nidulans* FGSC A4 (EAA59763), *E. nidulans* (P40920), *A. oryzae* (BAD02411), *E. dermatitidis* (AAL84791) were 65%, 63%, 63%, 59%, and 54%, respectively. Conserved region of *sHsp*s is not continue, N-terminal region is not conserved and C-terminal region is quite conserved (Fig. 2).

The number of amino acid of *sHsp*s has a large variation: 228 amino acids in *N. crassa*, 181 amino acids in *A. nidulans*, 180 amino acids in *A. nidulans* FGSC A4, 166 amino acids in *A. oryzae*, 187 amino acids in *E. dermatitidis*, and 202 amino acids in *C. globosum*. *Hsp*s from *N. crassa* and A. *oryzae* have the most difference in the number of amino acid (62 amino acids). All of above described proved that the amino acid sequence of *sHsp*s from different fungi are not very conserved, and there are only 42 conserved amino acids in multiple sequence alignment (Fig. 2).

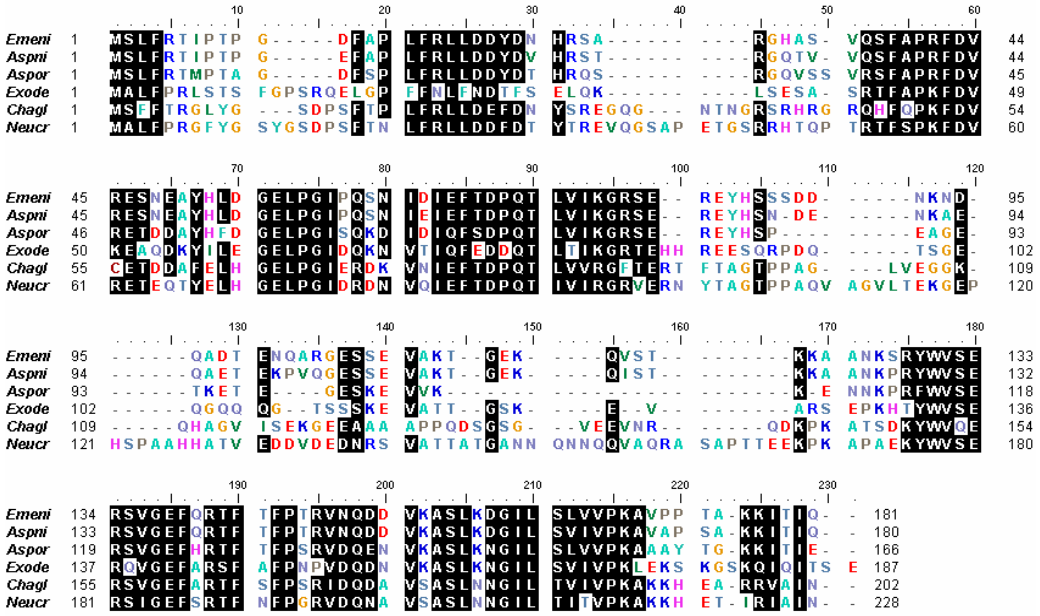

Fig.2 Multiple sequence alignment of *sHsp*s in difference fungi

Note: Emeni is *E. nidulans*, Aspni is *A. nidulans;* FGSC A4, Aspor is *A. oryzae*, Exode is *E. dermatitidis*, Chagl is *C. globosum*, Neucr is *N. crassa*

To investigate the relationship of *Hsp* with different plants, the molecular phylogenic tree was drown by Clustal program. The tree also showed that *C. globosum Hsp* protein was most close with *N. crassa Hsp* protein (Fig.3).

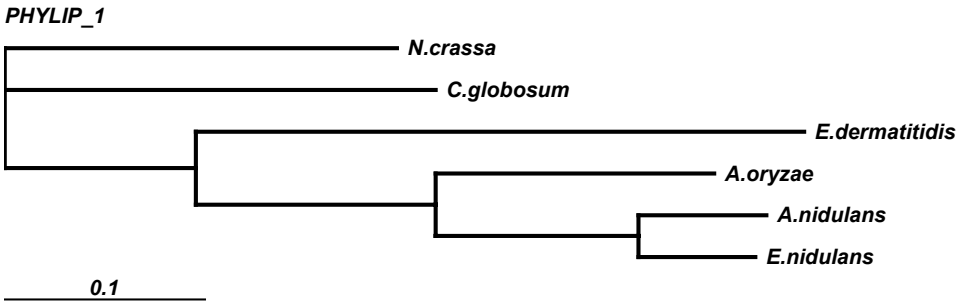

Fig.3 Molecular phylogenic tree of the amino acid sequences of the plant *Hsp* family.

Cloning of *Hsp* 22.4 gene and construction of recombinant vector

Hsp 22.4 gene was amplified from cDNA of *C. globosum* by PCR. Result of agarose gel electrophoresis showed a band about 630 bp was amplified, which consistent with expectation (Fig.4), DNA sequencing showed that the PCR product was *C globosum Hsp* 22.4 gene.

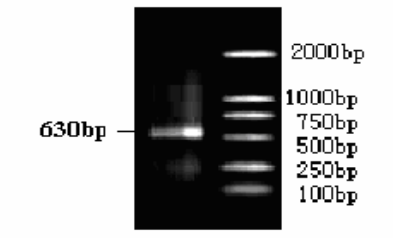

Fig.4 Cloning of *Hsp* 22.4 gene by PCR

Double enzyme digestion shows that the recombinant vector pGEX-HSPb has a 606-bp target product, while pGEX-HSPa has not this product (Fig. 5), which implies that target gene is inserted correctly into pGEX-HSPb and the result of DNA sequencing shows there is no mutant in recombinant vector.

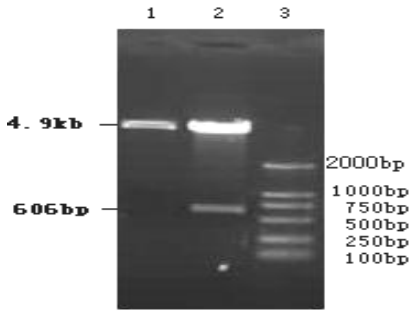

Fig. 5 Double enzyme digestion

Note: 1: pGEX-HSPa, 2: pGEX-HSPb, 3: DNA Marker DL2000

Expression of recombinant pGEX-HSPb

The fused protein have been expressed successfully in *E. coli* BL21 (DE3) by SDS-PAGE (Fig. 6), and the molecular mass of fused protein is about 50 kD, while the molecular mass of unfused protein in control (pGEX-4T-2) is about 28 kD. The result is consistent with expectation, showing that the *Hsp*22.4 gene had been expressed in *E.coli*. Further analysis showed the content of recombinant fused protein accounted for about 40% of total protein.

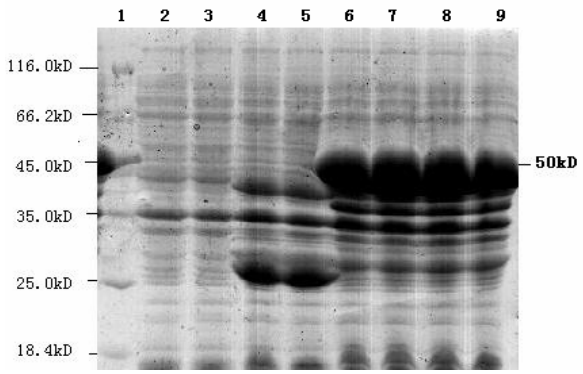

Fig. 6 SDS-PAGE analysis of recombinant protein expressed in *E. coli* BL21

Note: 1: Protein marker; 2: *E. coli* BL21/pGEX-4T-2 as control (not induced); 3: *E. coli* BL21/pGEX-HSPb as control (not induced), 4, 5: *E. coli* BL21/pGEX-4T-2 as control (induced 3 h, 6 h); 6, 7, 8, 9: *E. coli* BL21/pGEX-HSPb (induced 3 h, 4 h, 5 h, 6 h)

Discussion

Multiple sequence alignment indicates that *sHsp*s are overall variability in amino acid sequences. The research of Plesofsky *et al*. (2002) showed that the *Hsp*30 gene sequence is overall variable, and its conservative region is discontinuous, and has discrete regions of conserved sequence that are involved in structural organization, as well as nonconserved regions that may perform similar roles in each protein. They also held the views that the conserved alpha-crystallin domain can be divided into N-terminal and C-terminal subdomains that interact strongly with one another. To understand the role of the N-terminal region, Usui *et al*. (2004) constructed N-terminal truncation mutants of *StHsp*14.0, the *sHsp* from *Sulfolobus tokodaii* strain 7. The results revealed that the mutants exhibited reduced chaperone activity for the protection of 3-isopropylmalate dehydrogenase from thermal aggregation. Chaperone activity and secondary

structure of *Hsp*30 from *Xenopus* can be inhibited by phosphorylation or mutagenesis of the C-terminal end (Heikkila 2003).

As an important factor of endogeneity cytoprotection, the research on *sHsp*s has many important values in theory and application. The promoter regions of *Coriolus versicolor* contain the factors of consensus heat shock, a xenobiotic-response element, a stress-response element, and a metal-response element. In the stationary phase of growth, *Saccharomyces cerevisiae* can induce high-efficient expression of *Hsp*30 and can also make this enzyme keep activity in the anaphase of fermentation (Riou *et al*. 1997). A marine bacterium (*Vibrio harveyi*) contains a single *sHsp*s which is strongly induced by heat shock and reacts with the anti-IbpA/B serum (Klein *et al*. 2001). Transcripts of a heat-shock protein in *Populus euphratica* Oli accumulated at 1.5 h, after adding 300 mmol·L^{-1} NaCl to the culture medium (Gu *et al*. 2004). An expression vector containing the open reading frame of the *Hsp*30C gene from *Xenopus* was expressed in *E. coli.* These bacterial cells displayed greater thermo-resistance than wild type or plasmid-containing cells (Fernando *et al*. 2000). The study of *Hsps* of *C. globosum* has not been reported now. The cloning of *Hsp*22.4 gene from *C. globosum* and its expression in *E. coli* will lay a foundation for further studying its stress-resistant mechanism and exploring its function in *C. globosum*. Meanwhile, *Hsp*22.4 gene from *C. globosum* will be a desirable target gene in genetic engineering for culturing stress-tolerance plant and microorganism.

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